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METHODS OF COLLECTION AND ANALYSIS OF PLANKTON AND PERIPHYTON SAMPLES IN THE WATER POLLUTION SURVEILLANCE SYSTEM

Cornelius I. Weber

Federal Water Pollution Control Administration
Cincinnati, Ohio

July 1966

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Applications and Development Report

No. 19

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Federal Water Pollution Control Administration
Department of the Interior

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PREFACE

Dr. Weber is in charge of plankton and periphyton studies and serves as Assistant Chief of Aquatic Biology in the Water Pollution Surveillance System Laboratories at Cincinnati, Ohio. Since his association with this activity in September 1963, he has conducted a number of evaluations of methods and techniques. In addition, he has developed sampling and analysis procedures for periphyton. Studies of the periphyton can be especially useful in detecting influences of specific pollution sources when samplers are appropriately located. The Aquatic Biology program is directed by Mr. Joseph B. Anderson.

The methods for analysis of plankton samples described in this report are essentially those developed by Dr. Louis G. Williams while he was in charge of the plankton program during the period October 1958 - December 1962. A significant change subsequently introduced in plankton analysis, however, was the use of centrifugation rather than settling for the concentration of diatoms.

A. W. Breidenbach, Ph.D.
Assistant Chief for Laboratories
Division of Pollution Surveillance

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METHODS OF COLLECTION AND ANALYSIS OF PLANKTON AND PERIPHYTON SAMPLES IN THE WATER POLLUTION SURVEILLANCE SYSTEM

I. Plankton

A. Collection

Plankton samples are obtained from water plant intakes or directly from lakes or rivers at a depth of 2 to 15 feet. The sample volume varies from 1 to 3 liters, depending on the types of analyses to be performed. One liter is sufficient for a phytoplankton Sedgwick-Rafter count and diatom species analysis; a 3-liter sample is collected if a zooplankton count is also to be made. The narrow-mouth polyethylene sample bottles are shipped in individual, cushioned, fiberboard cartons (Figure 1), and contain MERTHIOLATE preservative when mailed to the station. The bottles are accompanied by a sampling date reminder, and a tag (Appendix) for the sampling data.

B. Preservation

The MERTHIOLATE preservative stock solution is prepared by dissolving the following in 1 liter of distilled water:

1.0 gram of MERTHIOLATE (sodium ethyl-mercury thiosalicylate)

1.0 ml of aqueous saturated Iodine-KI solution prepared by dissolving 60 grams of KI and 40 grams of I_2 in 1 liter of distilled water

1.5 grams of Borax (sodium borate)

Figure 1. Plankton Sample Bottles and Shipping Containers.

To each plankton sample bottle shipped from our laboratory sufficient volume of stock solution is added to provide 36 mg of MERTHIOLATE, 54 mg of Borax, and 1.3 mg of Iodine per liter of water when the bottle is filled with sample. This preservative effects excellent color retention and causes no morphological distortion. Although sterility is not achieved at this concentration of MERTHIOLATE, samples may be stored on the shelf at least 1 year without deterioration. Phytoplankton growth is arrested at MERTHIOLATE concentrations as low as 2 mg per liter,

but gradual bacterial deterioration of the plankton occurs at less than 10 ppm. The cost of preserving a 3-liter sample is approximately $0.02.

C. Sedgwick-Rafter Phytoplankton Analysis

The plankton sample is mixed by inverting the sample bottle no fewer than seven times, and a 50- to 100-ml volume is poured immediately into a small beaker. The contents of the beaker are well mixed by repeatedly filling and discharging a 1-ml pipette. Then, without delay, the pipette is filled with sample, and the liquid is directed diagonally across the bottom of a Sedgwick-Rafter cell. (One-half of the chamber is filled from each of the opposite corners - see Figure 2.) As the chamber fills, the cover glass rotates on the water film and becomes aligned with the chamber. Excess water in and around the chamber is removed with a blotter. After it is filled, the counting chamber is placed on the microscope stage and allowed to stand 15 minutes to permit the algae to settle to the bottom.

If the phytoplankton are obscured by silt, a 1-ml aliquot of sample is diluted 5 to 10 times with tap water and the cell is refilled.

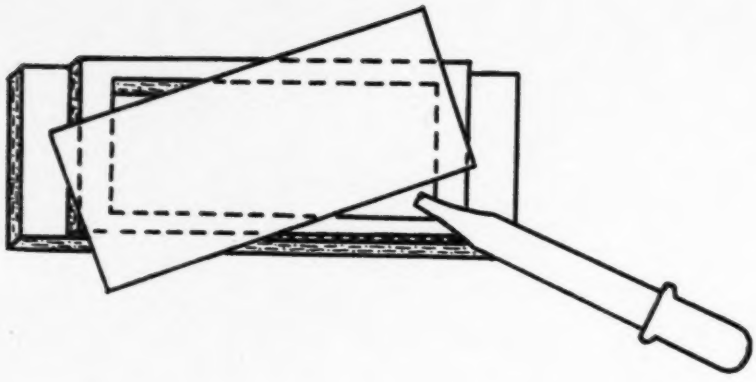

Figure 2. Filling the Sedgwick-Rafter Cell.

The count is made by scanning two strips across the cell (Figure 3) at 200X, each strip being the width of a Whipple grid (approximately 0.45 mm). Two longitudinal strips include an area approximately twice 0.45 X 50 mm, or 45 mm^2. Since the chamber is 1 mm deep, the total volume examined would be 0.045 ml. The bottom of the cell is divided into five sections by transverse lines used as reference marks when scanning.

As the non-diatoms are counted, they are identified to species, if possible, and tallied on a bench sheet (Appendix) in one of the following categories: coccoid blue-green, filamentous blue-green, coccoid green, filamentous green, green flagellate, or other flagellated algae. Each solitary cell, or natural group (colony) of cells, is tallied as one unit. If, during a count, 100 or more of a given alga are tallied in the first section of the Sedgwick-Rafter cell

(a tenth of the total scanned area), the tally for this organism is immediately converted to units per ml and the alga is disregarded for the rest of the count. This procedure is followed whenever 100 or more of any organism are tallied before the count is nine-tenths complete.

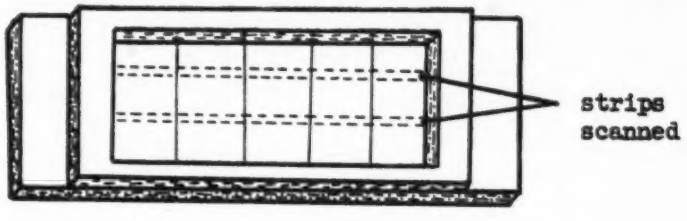

Figure 3. Sedgwick-Rafter Strip Count.

A cell count (not a unit count) is made of the diatoms, which are tallied as live centrics, centric shells (empty frustules), live pennates, or pennate shells (empty frustules). In practice, frustules containing any part of a protoplast are tallied as live.

If a sample contains organisms so small they are difficult to identify at 200X, a 10-ml aliquot is centrifuged and a wet mount is examined at 970X. Those forms that cannot be identified with certainty are arbitrarily assigned to the category considered most appropriate by the examining biologist.

D. Diatom Species Proportional Analysis

Diatom species proportional counts are made from permanent slides prepared from plankton concentrates obtained by centrifuging aliquots of the samples. Routinely, a 100-ml aliquot of a thoroughly mixed sample is centrifuged 20 minutes at 1000 G, and the supernatant water is decanted with a suction tube. Tests have shown that the diatoms are quantitatively removed from the aliquot by centrifugation. The plankton concentrate is poured into a disposable 3-dram vial, and the station number, name, and date are written on the side of the vial with a black, felt, marking pen. The vial is then allowed to stand at least 24 hours before further processing.

All but a few milliliters of water are then withdrawn from the vial with a suction tube. If the water contains more than 1 gm of dissolved solids per liter, as in the case of brackish water or marine samples, the salt crystals will obscure the diatom frustules on the finished slides. In this case, the concentration of salts is reduced by refilling the vial with distilled water, resuspending the plankton, and allowing the vial to stand 24 hours before removing the supernatant liquid. The dilution is repeated several times if necessary.

The diatom slides are prepared as follows:

1. The plankton concentrate in a vial is thoroughly mixed with a disposable pipette, and several drops are delivered to a No. 1 circular, 18-mm coverglass. Twenty to 30 samples are usually processed at one time by placing the coverglasses on a piece of sheet metal, 5 X 10 X 1/8 inches.

2. The samples are dried on a hotplate at 95°C. (Caution: overheating may cause splattering and cross-contamination of the samples.)

3. When the material has dried, the coverglasses are examined to determine if there is sufficient material for a diatom count.

4. Steps No. 1 and 2 are repeated one or more times, depending on the density of plankton and sediment in the vial.

5. The metal plate bearing the coverglass is then heated at approximately 1000°F for 30 minutes. (It is best to have two hotplates; a low-temperature plate for drying, and a high-temperature plate for incinerating.)

6. Using a No. 3 pencil, the frosted end of a 25- X 75-mm microscope slide is labeled with the name of the river or lake, the station name and number, and the sampling date (Figure 4).

7. The labeled slide is then placed on a moderately warm hotplate (250°F), a drop of Hyrax mounting medium (R. I. 1.65) is placed in the center, and the slide is heated until the hyrax

solvent (xylene) is driven off. When the solvent has evaporated, the slide is ready to receive the coverglass. One can determine when the solvent is gone by periodically touching a dissection needle to the Hyrax on the slide and allowing the needle to cool. The Hyrax will become hard and brittle upon cooling. (The same hotplate used to dry the plankton concentrate on the coverglass is used to prepare the Hyrax on the slide.)

8. Grains of sand or other large objects on the coverglass should be removed with a dissection needle. The oil immersion objective has a very small working distance, and the slide may be unusable if this material is not removed.

9. While the coverglass and slide are still hot, the coverglass is grasped with a tweezer, inverted, and placed on the drop of melted Hyrax on the slide. Slight pressure is applied to the coverglass with a cylindrical object (e.g. pencil eraser), and the coverglass is centered on the slide. It may be necessary to add Hyrax at the margin of the coverglass.

10. Some additional bubbles of solvent vapor may appear under the coverglass when it is placed on the slide. When the bubbling ceases, the slide is removed from the hotplate and placed on a firm, flat surface. Pressure is immediately applied to the coverglass as described in step No. 9 and

maintained until the Hyrax cools and hardens (about 5 seconds). Bubbles in the Hyrax are pressed out by moving the pencil eraser around the edge of the coverglass.

11. A protective coating of clear lacquer is sprayed on the frosted end of the slide.

12. The excess Hyrax is scraped from around the coverglass.

To begin the diatom count, the slide is scanned to locate an area that is relatively free of silt and contains a moderate density of diatoms. Lateral strips the width of the Whipple grid are then examined (Figure 4), and all diatoms within the borders of the grid are counted and identified to species (see bench sheet in Appendix).

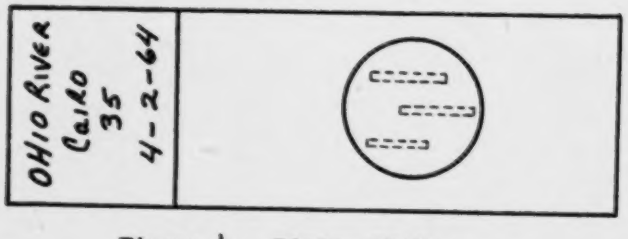

Figure 4. Diatom Slide.

If, before the count is completed, the lateral movement of the slide brings the grid image to the edge of the coverglass or to an area of dense sediment, the slide is shifted up or

down and the count is continued in another strip. Small cell fragments are ignored.

In a typical diatom analysis, 200 to 300 diatom cells are identified and tallied on the bench sheet. However, if the slide has a scarcity of diatoms, dictated by the lack of material in the sample, the analysis is limited to the number of cells encountered in 45 minutes of scanning. If the generic or specific determination of a diatom cannot be made, it is recorded as unknown. When the count is completed, the tallies are totaled, and the percentages of the four most abundant species are calculated and recorded.

If the plankton counts are less than 500 per ml, the centrifugation method may not provide enough diatom material to prepare a countable slide. In this case the diatoms may be concentrated from a larger volume of sample (1 liter) by allowing them to settle out. However, caution must be exercised in the use of this method because it does not quantitatively remove diatom cells smaller than 10μ in diameter in less than 14 days' settling; consequently, this method can only be used safely and economically for samples with large forms of diatoms.

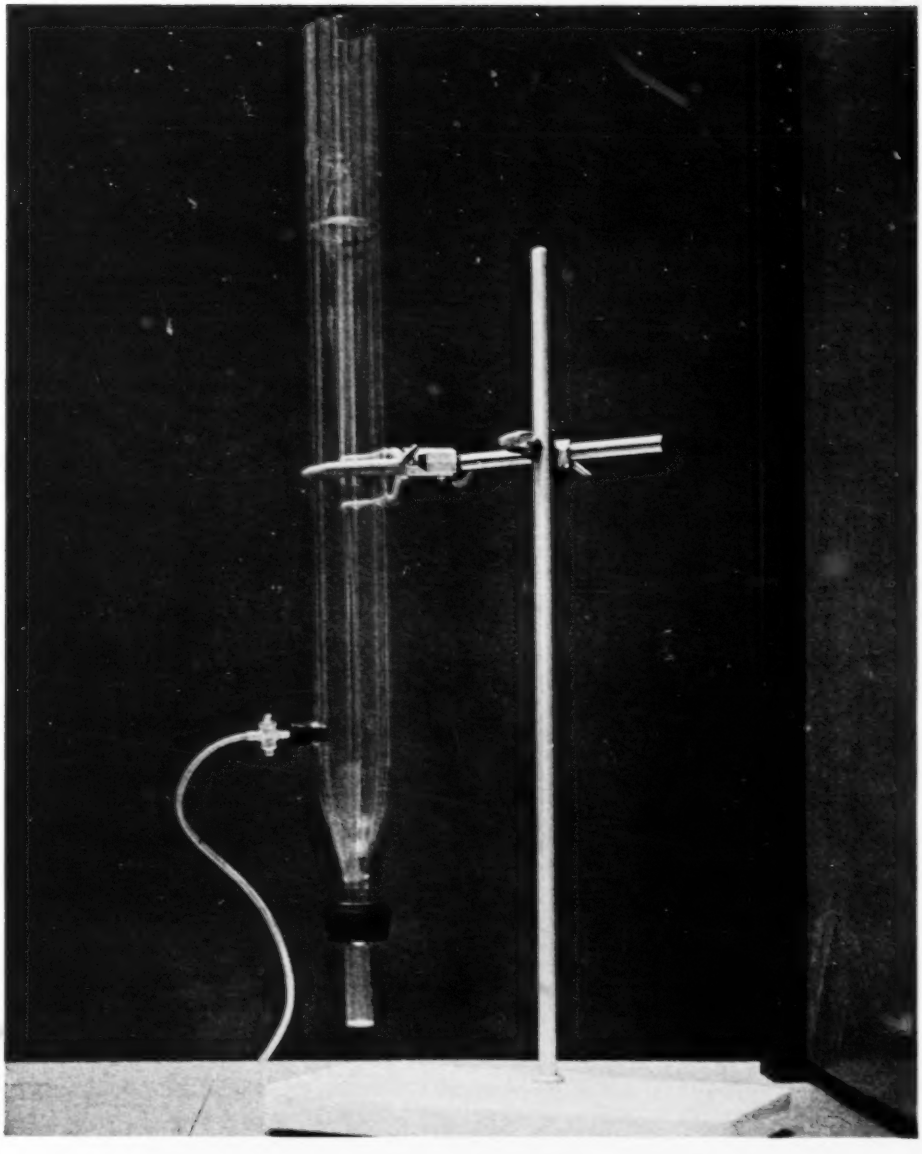

Figure 5. Settling Tube.

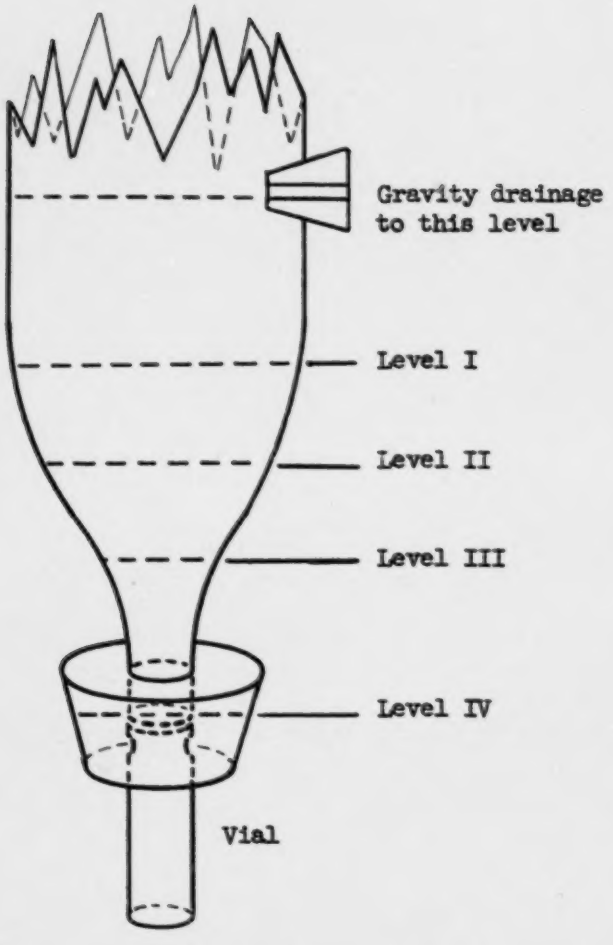

Figure 6. Lower Portion of a Settling Tube.

In the settling method, the sample is thoroughly mixed and approximately 1 liter is poured into a cylinder (Figure 5). After 48 hours the cylinder is emptied through a side port, the drain valve and stopper are removed, and the water is lowered to level I (Figure 6) by use of a small suction tube introduced through the drain port. The cylinder is then swirled to loosen the deposits on the shoulder at the lower end and allowed to stand 1 hour to permit the plankton to resettle. The water is then lowered to level II, and the cylinder is again swirled and allowed to stand 1 hour. The process is repeated until the sediment has been deposited in the vial. The vial is then removed, and a diatom slide is prepared as described above.

E. Zooplankton Analysis

Rotifers and micro-crustacea are quantitatively removed from the samples by settling 1 liter of sample 24 hours in the cylinder as described in the preceding paragraph. If more than a half inch of sediment collects in the vial, it may be necessary to dilute the concentrate before the counts can be made. The turbidity in sample vials containing lesser amounts of solids can be removed by using the following method:

a. After standing 15 minutes, three-quarters of the water above the sediment is withdrawn with a suction tube.

b. The vial is refilled with tap water, inverted several times, and allowed to settle 15 minutes.

c. Steps a and b are repeated as many times as necessary to obtain a countable sample.

The zooplankton concentrate is then brought to a volume of 8 ml, mixed well, and the entire sample is placed in a counting chamber 80 X 50 X 2 mm (Figure 7), using the same technique described for filling a Sedgwick-Rafter cell.

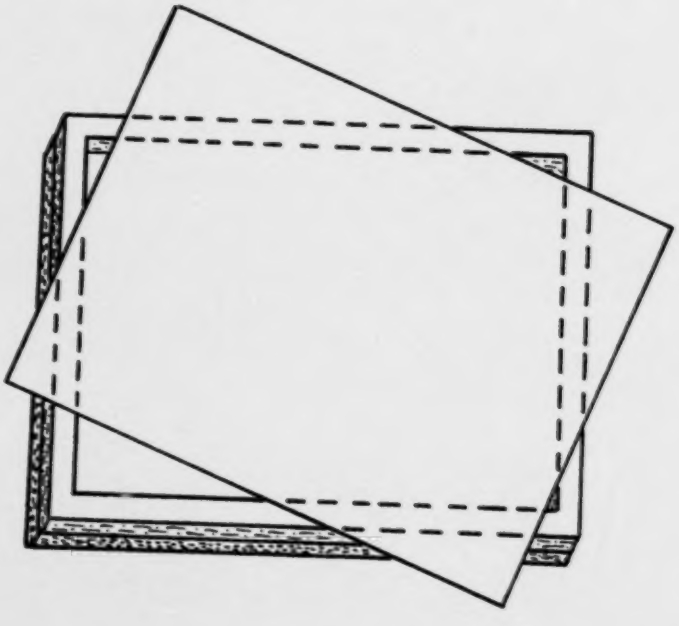

Figure 7. Zooplankton Counting Chamber.

Rotifers

Ten strips across the chamber are scanned at 100X (a fifth of the chamber), and the rotifers are identified to genus. If no rotifers are encountered in the strips, a zero count is recorded. If a tally of 100 is reached for any genus before the count is nine-tenths complete, the tally of that genus is discontinued at the end of the strip being counted, and that count is multiplied by a factor to convert it to organisms per liter.

Crustacea

Nauplii are enumerated at the time of the rotifer count. Adult copepods, cladocera, and other large forms are enumerated under a binocular dissecting microscope at 20X by scanning the entire contents of the zooplankton cell. Crustacea are identified to genus only.

II. Periphyton

A. Collection

The sampler consists of a styrofoam float approximately 12 X 12 X 2 inches, which supports a central plexiglass cradle holding 1- X 3-inch glass microscope slides (Figure 8). Generally, two slides are exposed at each station for 2 weeks. However, the exposure time may vary, depending upon arrangements made with local cooperating personnel. At the end of the exposure period, the slides are removed from the sampler, placed in a 3-ounce bottle containing approximately 70 ml of 5% formalin, and shipped to our laboratory. A bottle containing preservative, a sample data tag (see Appendix), and clean slides are mailed to the station in advance of the collection of the sample (Figure 9). The mailing container is supplied with a franked, return address label.

B. Preservation

A 5% formalin solution is prepared by diluting technical grade formaldehyde solution (37% HCHO) with distilled water.

C. Sample Preparation

With a razor blade, the periphyton is scraped from the slides into the 3-ounce sample bottle, and preservative is

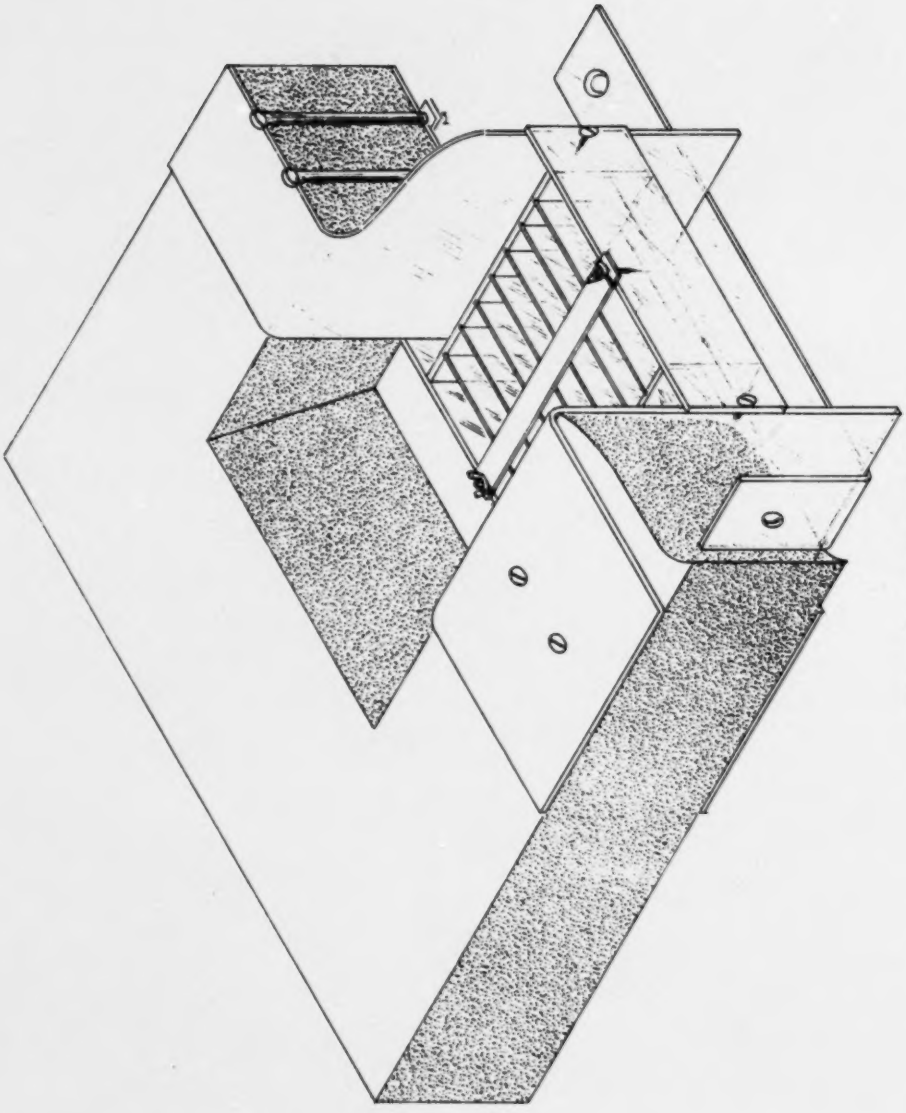

Figure 8. Periphyton Sampler.

added to bring the total volume to 90 ml. At this time, 5 to 8 ml of the sample is poured into a disposable 3-dram vial and set aside for diatom slide preparation.

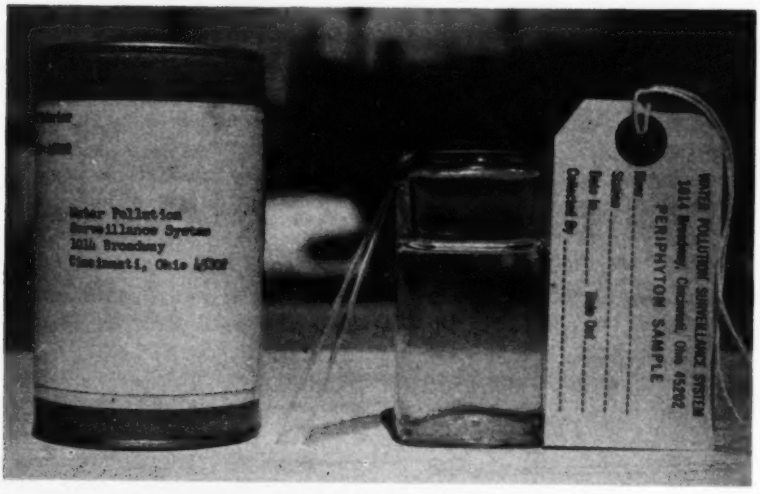

Figure 9. Periphyton Sample Bottle.

D. Sedgwick-Rafter Cell Analysis

After thoroughly mixing the sample by repeatedly filling and discharging a straight-sided pipette (inside diatmeter 3 mm) in the bottle, 1 ml is transferred to a Sedgwick-Rafter cell, and a strip count is made. The counting procedure is same as that outlined in the plankton section, except that a cell count is made of all organisms (see bench sheet in Appendix). If the organisms are too concentrated to permit a direct count, a 1-ml aliquot is diluted to 5 ml, and the material is placed in the Sedgwick-Rafter cell. Further dilution is occasionally

necessary. The scrapings may contain clumps of cells, even after the sample is thoroughly shaken. This may result in a more uneven distribution of material in the counting cell than occurs with the plankton samples, but it cannot be entirely avoided.

E. Diatom Species Proportional Analysis

The same procedures (and bench sheet) used for the preparation and counting of plankton diatoms are used to process the periphyton samples, except that a chemical treatment is frequently used to separate the aggregates (colonies) of diatoms into individual cells. In this case the intercellular gelatinous matrix is digested with the oxidant, potassium persulfate ($K_2S_2O_8$). Prior to the oxidation step, the formalin solution is decanted from the diatom sample vial with a suction tube. A 5% $K_2S_2O_8$ solution is added, and the sample is heated to 95°C for at least 30 minutes. The sample is then allowed to cool and settle for 24 hours. The $K_2S_2O_8$ solution is decanted with a suction tube, and the vial is refilled with distilled water and allowed to stand 24 hours. A minimum of three changes of distilled water are necessary to remove enough of the residual salt from the sample so that a crystalline layer does not form when the material is dried on the coverglass.

Appendix

1. Plankton Tag and Sampling Reminder.
2. Periphyton Tag.
3. Sedgwick-Rafter Plankton Bench Sheet.
4. Diatom Bench Sheet.
5. Periphyton Sedgwick-Rafter Bench Sheet.

WATER POLLUTION SURVEILLANCE SYSTEM
1014 Broadway, Cincinnati, Ohio 45202
PLANKTON SAMPLE

River_____
Station_____
Date_____
Collected by_____

NOTICE

Whenever possible, plankton samples should be collected during the first full week of each month. This sample bottle should be filled and shipped during the week of

_____

Extra bottles that accumulate because of missed samples should be returned empty to the Water Pollution Surveillance System (formerly the National Water Quality Network) in Cincinnati.

WATER POLLUTION SURVEILLANCE SYSTEM
1014 Broadway, Cincinnati, Ohio 45202
PERIPHYTON SAMPLE

River_____
Station_____
Date In_____ Date Out_____
Collected By_____

SEDGWICK-RAFTER DATA

River or Lake _____ Date Analyzed _____ Station No. _____

Station _____ Analyzed by _____ Date Collected _____

State _____

GENUS	SPECIES	TALLY	C/ML.	TOTALS
	Total coccoid blue-green algae per ml.			
	Total filamentous blue-green algae			

ANIMAL ANALYSIS

CODE	ORGANISM	TALLY	C/LITER
	ROTIFERA		
11	Keratella		
02	Brachionus		
17	Polyarthra		
21	Synchaeta		
22	Trichocera		

24

DIATOM ANALYSIS

River _____ Station _____ State _____

Live Centrics _____ Dead Centrics _____

Live Pennates _____ Dead Pennates _____

Total Live _____ Total Dead _____

S-R Count _____

Station Number _____

Date Collected _____

Analyzed by _____

Date Analyzed _____

Counting Time _____

Species	Total	%
Coscinodiscus		
Cyclotella		
Meneghiniana		
Melosira		
ambigua		
granulata		
distans		
Rhizosolenia		
Stephanodiscus		
hantzschii		
invisitatus		
astrea minutula		
Other centrics		
Achnanthes		
Amphiprora		
Amphora		
Asterionella formosa		
Caloneis		
Cocconeis		
Cymatopleura		
Cymbella		
Diatoma vulgare		
Diploneis smithii		
Epithemia		
Eunotia		
Fragilaria crotonensis		
construens		
Frustulia		
Gomphonema		
Gomphoneis		
Gyrosigma		
Meridion circulare		
Navicula		
Nitzschia		
Pinnularia		
Pleurosigma		
Rhoicosphenia curvata		
Stauroneis		
Rhopalodia		
Surirella		
Synedra		
ulna		
acus		
Tabellaria		
fenestrata		
flocculosa		

	FIRST	SECOND	THIRD	FOURTH	Percent others
Code					
%					

No. species _____

Remarks:

Total count _____

NL-C-15
(6-66)

(9-65)

25

Periphyton Data

River or Lake _____ Inclusive Dates _____

Station _____ Date Analyzed _____

State _____ Analyzed by _____

CODE	ORGANISM	Tally	c/ml	c/mm²	c/mm²
	Total coccoid blue-green algae				
	Total Filamentous blue-green algae				
	Total coccoid green algae				
	Total Filamentous green algae				
	Total green flagellates				
	Other coccoid algae				
	Other pigmented flagellates				

Centrics	c/ml

Pennates	c/ml

Most abundant algae

Diatoms

	c/ml	c/mm²
Centric shells		
Live centrics		
Total live centric diatoms		
Pennate shells		
Live pennates		
Total live pennate diatoms		

S-R Factor _____

TOTAL LIVE ALGAE (cells/mm²)

Preservative _____

No. slides collected _____

Area scraped _____

Scrapings diluted to _____ ml

First check _____ Wash. sheet _____

Recorded _____ Wash. sheet checked _____

REMARKS: